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Patent
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TECH CENTER 1600/2900

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Sub C1 100. (Amended) A method of regulating expression of a desired gene in an animal, said method comprising:

administering to said animal a pharmacological dose of a ligand which binds to a mutated steroid receptor superfamily ligand binding domain,

wherein said animal contains:

(a) a first nucleic acid cassette which comprises a promoter transcriptionally linked to a mutated receptor protein coding sequence,

wherein said mutated receptor protein coding sequence comprises a nucleic acid sequence encoding a mutated receptor protein which regulates the transcription of a molecular switch promoter, and wherein said mutated receptor protein comprises:

a DNA binding domain which binds said molecular switch promoter;

a mutated steroid hormone receptor superfamily ligand binding domain distinct from a naturally occurring ligand binding domain;

a transactivation domain which causes transcription from said molecular switch promoter when said mutated receptor protein is bound to said molecular switch promoter and to an antagonist for a nonmutated receptor protein; and

(b) transcriptionally linked to said molecular switch promoter, a nucleic acid encoding a desired protein in a second nucleic acid cassette; wherein administration of said ligand regulates expression of said desired gene in said animal.

Sub D1 101. The method of claim 100, wherein the mutated steroid hormone superfamily receptor ligand binding domain is selected from the group consisting of estrogen, androgen, Vitamin D, COUP-TF, cis-retinoic acid, Nurr-1, thyroid hormone, mineralocorticoid, glucocorticoid-alpha, glucocorticoid-beta, and orphan receptor ligand binding domains.

102. (Amended) The method of claim 100, wherein the mutated receptor protein is comprised of a progesterone receptor with the native DNA binding domain replaced with a GAL-4 DNA binding domain.

103. (Amended) The method of claim 100, wherein the nucleic acid encoding said desired protein is transcribed to produce an mRNA molecule that is translated to produce a protein after the animal is given a dose of a ligand which binds to the mutated steroid hormone receptor superfamily ligand binding domain.

104. (Amended) The method of claim 100, wherein the first nucleic acid cassette and the second nucleic acid cassette in said animal are on separate plasmids.

105. The method of claim 100, wherein the mutated steroid receptor comprises a non-native or modified DNA binding domain.

107. (Amended) The method of claim 100, wherein said animal is a mammal.

108. The method of claim 107, wherein said mammal is a human.

111. (Amended) The method of claim 100, wherein the molecular switch is linked to a nucleic acid cassette thereby forming a cassette/molecular switch complex and said complex is positionally and sequentially oriented in a vector such that the nucleic acid in the cassette is transcribed and translated in said target animal.

112. The method of claim 100, wherein the mutated steroid hormone receptor ligand binding domain includes an ecdysone ligand binding domain.

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113. The method of claim 100, wherein the mutated steroid hormone receptor ligand binding domain binds a compound selected from the group consisting of 5-alpha-pregnane-3,2-dione; 11 beta-(4-dimethylaminophenyl)-17 beta-hydroxy-17 alpha-propinyl-4,9-estradiene-3-one; 11 beta-(4-dimethylaminophenyl)-17 alpha-hydroxy-17 beta-(3-hydroxypropyl)-13 alpha-methyl-4,9-gonadiene-3-one; 11 beta-(4-acetylphenyl)-17 beta-hydroxy-17 alpha-(1-propinyl)-4,9-estradiene-3-one; 11 beta-(4-dimethylaminophenyl)-17 beta-hydroxy-17-alpha-(3-hydroxy-1 (Z)-propenyl-estra-4,9-diene-3-one; (7 beta,11 beta,17 beta)-11-(4-dimethylaminophenyl)-7-methyl-4',5'-dihydrospiro[ester-4,9-diene-17,2'(3'H)-furan]-3-one; (11 beta,14 beta,17 alpha)-4',5'-dihydro-11-(4-dimethylaminophenyl)-[spiroestra-4,9-diene-17,2'(3'H)-furan]-3-one.

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114. The method of claim 100, wherein the mutated steroid hormone superfamily receptor ligand binding domain is mutated to bind a compound selected from the group consisting of non-natural ligands, non-native hormones and anti-hormones.

115. The method of claim 100, wherein said DNA binding domain is replaced with a DNA binding domain selected from the group consisting of GAL-4 DNA binding domain, virus DNA binding domain, insect DNA binding domain and a non-mammalian DNA binding domain.

116. The method of claim 100, wherein said transactivation domain is selected from the group consisting of VP-16, TAF-1, TAF-2, TAU-2.

117. The method of claim 116, wherein said transactivation domain comprises a TAF-1 transactivation domain.

118. The method of claim 100, wherein said transactivation domain is a VP-16 transcription region and wherein said DNA binding domain is a GAL-4 DNA binding domain.

119. The method of claim 100, wherein said transactivation domain is a TAF-1 transcription region and wherein said DNA binding domain is a GAL-4 binding domain.

120. The method of claim 100, wherein said molecular switch is tissue specific.

121. The method of claim 120, wherein the tissue specificity of said molecular switch is controlled by selection of a tissue-specific transactivation domain.

122. The method of claim 120, wherein the molecular switch further comprises a tissue-specific cis-element.

123. (Amended) The method of claim 100, wherein said mutated steroid receptor results from a deletion of carboxy terminal amino acids in the ligand binding domain.

127. The method of claim 100, wherein said ligand is an endogenous ligand for said mutated steroid hormone receptor.

129. (Amended) The method of claim 100, wherein said ligand is 11 beta-(4-dimethylaminophenyl)-17 beta-hydroxy-17 alpha-propinyl-4,9-estradiene-3-one.

130. The method of claim 100, wherein the ligand is an antiprogestrone.

Sub 131. The method of claim 100, wherein said ligand requires conversion to an active form in an end organ.

132. The method of claim 100, wherein said ligand has a side chain which increases or restricts solubility, membrane transfer or target organ accessibility.

Bb 133. (Amended) The method of claim 101, wherein said mutated steroid receptor superfamily ligand binding domain is a Vitamin D ligand binding domain.

134. (Amended) The method of claim 133, wherein said mutated receptor is activated when bound by the ligand 24,25-dihydroxy-Vitamin D.

Please add the following new claims:

Sub C³ 135. (New) A method of regulating expression from a desired gene in an animal comprising:

Bb administering to an animal a pharmacologic dose of a ligand that activates a molecular switch encoded by a first expression cassette and results in regulated expression of a desired gene from a second expression cassette, wherein the molecular switch comprises a mutated steroid hormone superfamily receptor ligand binding domain capable of activation by the administered ligand but not by a native ligand for a corresponding wild type steroid hormone superfamily receptor ligand binding domain.

Sub 136. (New) The method of claim 135, wherein the mutated steroid hormone superfamily receptor ligand binding domain is derived from a steroid hormone superfamily receptor selected from the group consisting of:

estrogen; progesterone; glucocorticoid- α ; glucocorticoid- β ; mineralcorticoid; androgen; thyroid hormone; retinoic acid; retinoid X; Vitamin D; COUP-TF; ecdysone; Nurr-1 and orphan receptors.

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137. (New) The method of claim 136, wherein the ligand binding domain is a mutated progesterone ligand binding domain and the ligand is an anti-progestin.

138. (New) The method of claim 137, wherein the anti-progestin is selected from the group consisting of: RU 38486; Org31806; and Org 31376.

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139. (New) The method of claim 135, wherein the molecular switch comprises a mutated steroid hormone receptor superfamily ligand binding domain operably attached to a natural steroid hormone DNA binding domain.

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140. (New) The method of claim 135, wherein the molecular switch comprises a mutated steroid hormone receptor superfamily ligand binding domain operably attached to a DNA binding domain selected from the group consisting of: GAL-4 DNA binding domain; viral DNA binding domains; insect DNA binding domains; and non-mammalian DNA binding domains.

141. (New) The method of claim 135, wherein the molecular switch further comprises a transactivation domain distinct from a steroid hormone receptor superfamily transactivation domain.

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142. (New) The method of claim 135, wherein the regulated expression is up-regulation.

143. (New) The method of claim 135, wherein the regulated expression is down-regulation.